

# Calcium Chloride Extractable Cadmium as an estimate of Cadmium Uptake by Subterranean Clover

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## Abstract

Cadmium (Cd) may accumulate in soils which have been regularly fertilized with phosphate fertilizers which contain Cd originating in rock phosphate. Soil was taken from three sites in the wheatbelt of Western Australia which were estimated to have received different amounts of phosphate fertilizer over the past decade. The pH was adjusted with dilute HCl or CaCO<sub>3</sub>. No Cd was added experimentally. The concentration of Cd in the whole tops of *Trifolium subterraneum* cv Mt Barker grown in a glasshouse pot experiment increased from 0.2–0.8 µg g<sup>-1</sup> dry wt at pH 6.6–6.9 (1:5 0.01 M CaCl<sub>2</sub>) to 2–4 µg g<sup>-1</sup> at pH 4.1–4.2. The highest concentration of Cd in the plant tops at any particular pH occurred on the soil which had the highest concentration of P in the CaCl<sub>2</sub> extract. There was a linear relationship between the concentration of Cd in the whole tops of sub-clover and the concentration of Cd in the CaCl<sub>2</sub> extracts which was independent of site. The concentration of Cd in the CaCl<sub>2</sub> extracts was a function of pH and concentration of P in the CaCl<sub>2</sub> extract.

## Introduction

Cadmium (Cd) can enter the agricultural food chain via the disposal of sewage sludge on agricultural land, accumulation in soil from industrial pollution (Cartwright *et al.* 1976; Merry and Tiller 1978) or application of phosphate fertilizers (Williams and David 1973). Superphosphate in Australia may contain up to 50 µg g<sup>-1</sup> cadmium (Williams and David 1973), approximately 50% of which is water-soluble (Mann 1989). The Cd content of pasture species has been shown to increase with the total amount of superphosphate applied (Williams and David 1973) or when soil pH was decreased (Williams 1977; Tiller 1988). The concentration of Cd in plants grown on soils in which the pH ranged from strongly acid to slightly acid or alkaline was poorly correlated with soil Cd extracted by acid or EDTA (Symeonides and McRae 1977) or DTPA (Kuo 1990).

The objectives of this work were to examine the effect of pH on the concentration of Cd in subterranean clover grown on three acid soils from the Western Australian wheatbelt and to determine whether Cd in CaCl<sub>2</sub> soil extracts was a good measure of plant-available Cd.

## Materials and Methods

The concentration of Cd was measured in *Trifolium subterraneum* L. cv Mt Barker grown in soil from three sites which had been acidified or limed to give a pH range of approximately 4–7 (1:5 0.01 M CaCl<sub>2</sub>).

## Experimental Procedure

Sites on three farms in the Western Australian wheatbelt (Table 1) were sampled to a depth of approximately 10 cm after clearing the surface of plant debris. The soils were air-dried and sieved ( $\leq 2$  mm) immediately after collection. The sites differed in recent management histories. Site 1 was in the pasture phase of a long rotation, site 2 had been continuously cropped for the past 10 years and site 3 was in the crop phase of a short crop: pasture rotation.

**Table 1. Summary of soils**

The vicinities of the soil sites were: site 1, Meckering; site 2, Goomalling; site 3, Merredin

Site	Classifica- tion <sup>A</sup>	pH <sup>B</sup>	Bic- P <sup>C</sup> ( $\mu\text{g g}^{-1}$ )	PRI <sup>D</sup>	Ox. extract. <sup>E</sup> ( $\mu\text{g g}^{-1}$ )		Org. C <sup>F</sup> (%)	Particle size (%)		
					Al	Fe		Sand	Silt	Clay
1	Dy 5.51	4.4	15.8	1.43	178	261	1.0	90	4	6
2	Dy 5.51	4.4	23.5	0.75	175	155	1.0	88	4	7
3	Gn 2.21	4.8	20.6	4.43	285	687	1.3	80	3	17

<sup>A</sup> Northcote (1984).

<sup>B</sup> 1:5 0.01 M CaCl<sub>2</sub>.

<sup>C</sup> Bicarbonate extractable P (Colwell 1963).

<sup>D</sup> Phosphate retention index: P adsorbed by 1 g soil in 10 mL 0.01 M KCl initially containing 10  $\mu\text{g mL}^{-1}$  P as KH<sub>2</sub>PO<sub>4</sub> divided by the final concentration of P.

<sup>E</sup> Oxalate extractable P; 3 g soil 100 mL<sup>-1</sup> acid ammonium oxalate (Searle and Daly 1977).

<sup>F</sup> Yeomans and Bremner (1988).

Although precise fertilizer histories are not known, site 2 is estimated to have received more phosphate fertilizer than the other two sites for the past 10 years at least. There was no known environmental or industrial source of Cd near the collection sites or the buildings where the experiment took place.

The glasshouse experiment consisted of subterranean clover grown in pots in root-cooling tanks in a factorial array of 3 soils, 8 pH values, 2 sources of nitrogen (N<sub>2</sub> fixation and NH<sub>4</sub>NO<sub>3</sub>) with three replicates and has been described in detail elsewhere (Whitten and Ritchie 1991). The pH treatments were dilute HCl (range: 0.06–0.8 cmol H<sup>+</sup> kg<sup>-1</sup>), nil (deionized water) or precipitated CaCO<sub>3</sub> (1.25 and 2.5 g per 3 kg pot) followed by incubation at field capacity for 8 days at 40°C. Nutrient solutions supplying (mg kg<sup>-1</sup> soil) Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O (0.67), H<sub>3</sub>BO<sub>3</sub> (0.83), CuSO<sub>4</sub>·5H<sub>2</sub>O (5), ZnSO<sub>4</sub>·7H<sub>2</sub>O (10), MnSO<sub>4</sub>·H<sub>2</sub>O (15), CoSO<sub>4</sub>·7H<sub>2</sub>O (0.4), K<sub>2</sub>SO<sub>4</sub> (145), and KH<sub>2</sub>PO<sub>4</sub> [110 (soils 1 and 2) and 176 (soil 3)] were applied as solutions to the surface of the soil which was then dried and mixed. The N-fertilized treatments received 24 mg N kg soil as NH<sub>4</sub>NO<sub>3</sub> before sowing and at 10 day intervals thereafter. Plants were harvested at 5 weeks and were washed in deionized water to remove any adhering soil particles. No Cd was added experimentally and all reagents for pH adjustments and nutrients were analytical grade.

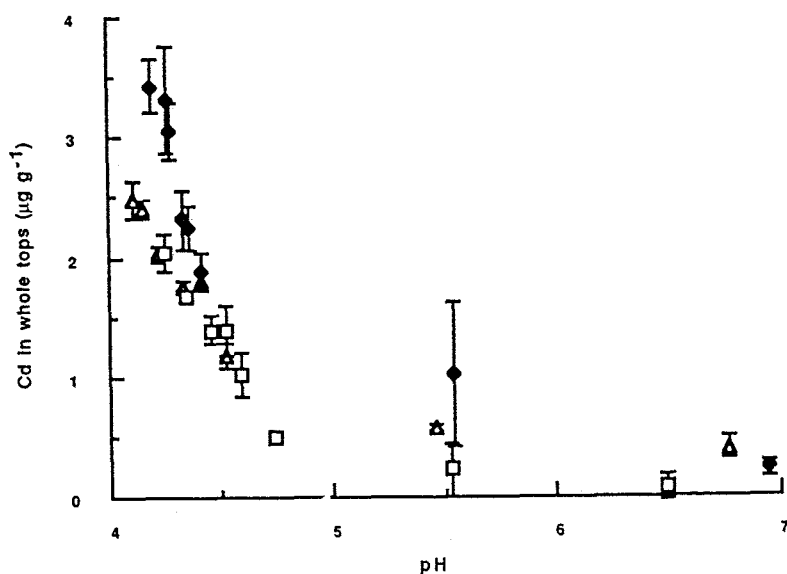
## Cadmium Analysis of Plants and Soils

All labware for Cd analysis was cleaned in 1 M HCl and 0.1 M Na<sub>2</sub>EDTA. The undiluted nitric/perchloric acid digest of the whole tops of the subterranean clover was analysed for Cd by flame atomic absorption spectrophotometry (FAAS) on a Perkin-Elmer 5000 atomic absorption spectrophotometer using background correction to eliminate interference from non-atomic absorption. The range of measured Cd in reference plant material (kale; Bowen 1975) was 0.64±0.12  $\mu\text{g g}^{-1}$ . Bowen (1975) reported the Cd concentration of this reference material to be 1.00±0.19  $\mu\text{g g}^{-1}$  when analysed by AAS, but suggested that this was an overestimation by 32% because of non-atomic absorption. Standards from a stock solution of Cd metal dissolved in HCl were made up in perchloric acid.

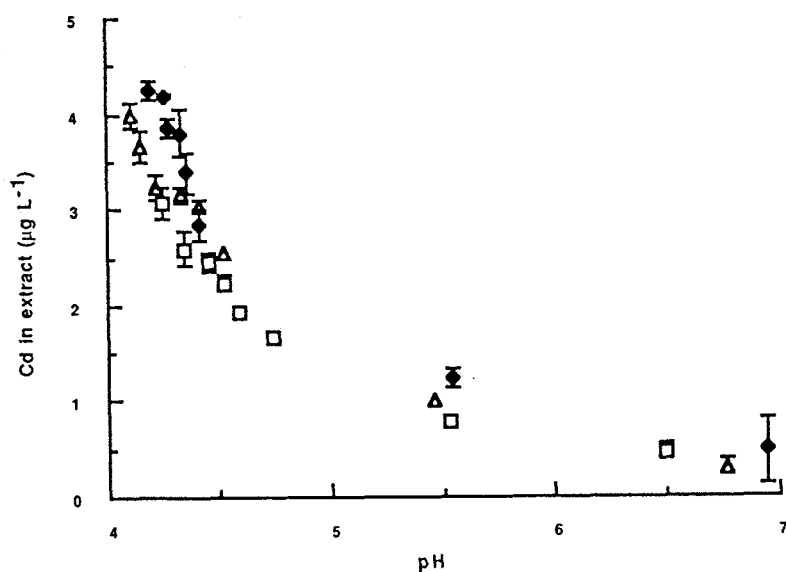
Subsamples of soil which had not received nutrients were shaken end-over-end for 16 h at 20°C in 1:5 0.01 M CaCl<sub>2</sub>, made up from a stock solution which had been purified

with dithizone extracted into chloroform to remove any trace amounts of Cd. The extracts were filtered through 0.4  $\mu\text{m}$  polycarbonate membranes (Nuclepore Corporation, U.S.A.). Cadmium in the filtrate was complexed with ammonium pyrrolidine dithiocarbamate (APDC) and extracted into 4-methylpentan-2-one (syn. methyl isobutyl ketone, MIBK) according to the method of Dudas (1974) in order to eliminate potential matrix effects during analysis. The MIBK extract was then analysed for Cd by flameless AA using pyrolytically coated graphite tubes in a Perkin-Elmer HGA 500 graphite furnace coupled to a PE 5000 with background correction. Standards were made up in double-deionized water immediately before complexing with APDC and extracting into MIBK.

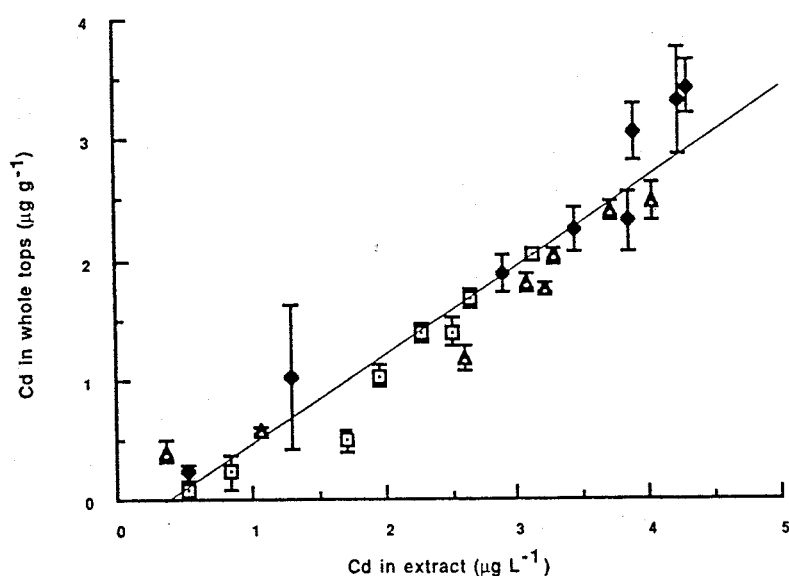
The pH and concentration of P in the  $\text{CaCl}_2$  extracts were measured with an Orion EA 940 pH meter and by the method of John (1970), respectively.



**Fig. 1.** Concentration of Cd ( $\mu\text{g g}^{-1}$ ) in the whole tops of subterranean clover plotted against pH in 1 : 5 extract in 0.01 M  $\text{CaCl}_2$ . Symbols ( $\Delta$ ) site 1, ( $\blacklozenge$ ) site 2 and ( $\square$ ) site 3. Error bars indicate standard errors.



**Fig. 2.** Concentration of Cd ( $\mu\text{g L}^{-1}$ ) plotted against pH in 1 : 5 extract in 0.01 M  $\text{CaCl}_2$ . Symbols ( $\Delta$ ) site 1, ( $\blacklozenge$ ) site 2 and ( $\square$ ) site 3. Error bars indicate standard errors.



**Fig. 3.** Concentration of Cd ( $\mu\text{g g}^{-1}$ ) in the whole tops of subterranean clover plotted against Cd ( $\mu\text{g L}^{-1}$ ) in 1:5 extract in 0.01 M  $\text{CaCl}_2$ . Symbols ( $\Delta$ ) site 1, ( $\blacklozenge$ ) site 2 and ( $\square$ ) site 3. Error bars indicate standard errors.

## Results

The concentration of Cd in the whole tops of the subterranean clover increased from 0.2–0.8  $\mu\text{g g}^{-1}$  dry wt at pH 6.6–6.9 to 2–4  $\mu\text{g g}^{-1}$  at pH 4.1–4.2 (Fig. 1). In the soil extracts, there was a corresponding increase in the concentration of Cd from 0.3–0.5  $\mu\text{g L}^{-1}$  to 3.0–4.3  $\mu\text{g L}^{-1}$  (Fig. 2). The relationship between the concentration of Cd in the whole tops of N-fixing subterranean clover and Cd in the  $\text{CaCl}_2$  extracts was

$$\text{Cd } (\mu\text{g g}^{-1}) = -0.301 + 0.775 \text{ Cd } (\mu\text{g L}^{-1}), \quad (1)$$

( $P < 0.01$ , adj.  $r^2 = 0.91$ ) and was independent of site (Fig. 3).

The highest concentration of Cd in the plant tops at any particular pH occurred on soil 2 which had the highest concentration of phosphate in bicarbonate and  $\text{CaCl}_2$  extracts. Although there was a significant ( $P < 0.01$ ) linear relationship between the concentrations of Cd and P in the  $\text{CaCl}_2$  extracts, the concentration of P accounted for only 24.5% of the variation in the concentration of Cd. The concentration of Cd in the  $\text{CaCl}_2$  extracts was best predicted by multiple linear regression against both the pH and the concentration of P in the  $\text{CaCl}_2$  extract. The regression equation was

$$\text{Cd } (\mu\text{g L}^{-1}) = 7.7348 - 1.146 \text{ pH} + 3.541 \text{ P (mg L}^{-1}\text{)}. \quad (2)$$

Both pH and P were significant ( $P = 0.0001$ ) and the adjusted  $r^2$  was 0.903.

Plants fertilized with  $\text{NH}_4\text{NO}_3$  contained slightly more Cd than those which relied on symbiotically fixed N. The relationship was

$$\text{Cd } (\mu\text{g g}^{-1}) \text{ N-fertilized} = 0.27 + 1.132 \text{ Cd } (\mu\text{g g}^{-1}) \text{ N-fixing}. \quad (3)$$

( $P = 0.0001$ , adj.  $r^2 = 0.92$ ).

## Discussion

Calcium chloride extractable Cd was a good indicator of the Cd content of subterranean clover. The concentration of Cd in the plant material and the  $\text{CaCl}_2$  extracts increased in a similar manner as the pH of the soil decreased.

### *Cadmium in Sub-clover*

The concentration of Cd in subterranean clover nearly doubled in each of the W.A. soils as the pH decreased from about 4.5 to 4 and was approximately halved as the pH increased from about 4.5 to about 5.5. The highest concentration of Cd in the whole tops of subterranean clover occurred in soil from site 2 which is estimated to have received the most phosphate fertilizer during the last 10 years. In Western Australia, therefore, acidification of soils used for pasture production could increase the uptake of Cd by plants and hence grazing stock. In addition, the continued use of phosphate fertilizers manufactured from rock phosphates containing Cd may increase the amount of Cd available to plants and animals.

Variation in concentration of Cd in plants could also be due to differences between soil type. Tiller (1988) reported twofold differences in the concentration of Cd in sub-clover growing in a red podzolic and a solodized solonetz over a pH range spanning 5.5–8.5 (1 : 5 water). These differences decreased with the addition of Cd.

The slightly higher concentration of Cd in the plant material from the N-fertilized treatment compared with the N-fixing treatment may be due to displacement of exchangeable Cd by  $\text{NH}_4^+$  or a slightly lower soil pH in the N-fertilized treatment. However, the final pH of the N-fertilized treatments was estimated to be only marginally lower than in the N-fixing treatments. This estimate was based on the differences in final soil pH under clover with different sources of N supplied at 350 mg at N per 3 kg pot under conditions similar to our experiment and with a soil of similar pH buffering capacity (Jarvis and Robson, 1983). It was calculated that production of 1 g shoot dry matter in our experiment would generate an excess of  $\text{H}^+$  in the N-fertilized treatment compared with the N-fixing treatment of approximately  $2 \times 10^{-6} \text{ mol L}^{-1}$  in the  $\text{CaCl}_2$  extract. This would be equivalent to a difference of approximately 0.04 pH units if the final pH was 4.5 in the N-fixing treatment.

### *Cadmium and Phosphorus in the Soil Extracts*

The concentration of Cd in the extracts was a function of pH and concentration of P in the extract. The correlation between the concentrations of Cd and P in the  $\text{CaCl}_2$  extract was poor in comparison with that for Cd extracted with 1 M HCl and P extracted with 0.5 M  $\text{H}_2\text{SO}_4$  (Williams and David 1973;  $r^2$  not stated therein), or between Cd extracted with 0.1 M EDTA at pH 6 and P in dilute  $\text{H}_2\text{SO}_4$  ( $r^2 = 0.55$ ; Merry 1988). In this work, the concentration of P in the  $\text{CaCl}_2$  extracts differed markedly between soils, but within each soil the effect of pH was much less pronounced than its effect on Cd.

In the  $\text{CaCl}_2$  extracts, exchangeable forms of Cd would go into solution, whereas specifically adsorbed Cd would not be readily displaced by Ca (Tiller *et al.* 1979). The relationship between the concentration of Cd and pH in the

extracts reflects increased adsorption of Cd with increased pH (Tiller *et al.* 1979). The amount of adsorbed Cd would also be influenced to some extent by the amount of adsorbed P. Over the pH range 4–7 (which corresponds with the pH range of the experiment described in this paper), adsorption of Cd by ferric hydrous oxides has been shown to increase as the amount of adsorbed P increased (Kuo and McNeal 1984). At the low pH of a 1 M HCl extract, relatively little Cd would remain adsorbed, regardless of the amount of adsorbed P or the pH of the soil.

### *Correlation between Cd in Soil Extracts and Plant Tops*

In acid soils, the concentration of Cd in the 1:5 CaCl<sub>2</sub> extracts at the ionic strength of the soil solution may be a more appropriate measure of Cd uptake by plants than that in acidic or buffered extracts because the pH of the 1:5 extract is similar to that in the soil. Alteration of the pH would affect the solubility and adsorption of Cd. Symeonides and McRae (1977) found that the concentration of Cd in radish tops grown on soils to which Cd had been added (pH 4.8–7.9; 1:2.5 water) was better correlated with the concentration of soil Cd extracted in unbuffered 1 M NH<sub>4</sub>NO<sub>3</sub> than in 1 M HCl or 0.00125 M EDTA. Kuo (1990) found that the concentration of Cd in whole tops of *Beta vulgaris* L. grown on a range of soils (pH 5–5.5; 1:2 water) to which different rates of sewage sludge had been added was only moderately well correlated ( $r^2 = 0.53$ ) with DTPA extractable Cd, and that plant Cd could increase as the pH decreased while DTPA extractable Cd remained constant.

### **Conclusions**

The Cd extracted in CaCl<sub>2</sub> extracts was able to predict Cd uptake by subterranean clover grown in agricultural soil from the three sites in which the pH had been altered to span the pH range of 4–7 in 1:5 0.01 M CaCl<sub>2</sub>. If similar results can be achieved over a similar soil pH range with a wider range of soil types and plant species, this test may assist in identifying sites at which Cd content of plants could be of concern. Soil acidity may be a contributing factor in the accumulation of Cd by grazing animals on soils to which large amounts of superphosphate containing trace amounts of Cd have been applied. However, soil ingestion could also be an important source of dietary Cd in grazing animals.

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